

SHORT COMMUNICATION

Pangamic Acid (Vitamin B₁₅, Pangametin, Sopangamine): Its Composition and Determination in Pharmaceutical Dosage Forms

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ABSTRACT

Pangamic acid is shown to be a mixture of variable composition. Criteria of identity and methods of analysis are described for five pharmaceutical dosage forms. Experimental results indicate that the products are not uniform in composition and that composition does not conform to label claims. No satisfactory preclinical drug application for any such preparation has so far been submitted to the Food and Drug Directorate.

SOMMAIRE

L'acide pangamique est un mélange dont la composition est variable. On décrit les critères de son identité et les méthodes d'analyse en ce qui concerne cinq formes pharmaceutiques. Les résultats expérimentaux indiquent que les produits n'ont pas une composition uniforme et que cette composition n'est pas conforme aux spécifications de l'étiquette. Jusqu'ici, aucune présentation préclinique pour de telles drogues n'a été soumise à La Direction des aliments et drogues.

IN 1951 Krebs *et al.*¹ reported the isolation of a crystalline compound C₁₀H₁₉NO₈ (Structural Formula I, Fig. 1) — called pangamic acid — from rice bran and apricot kernels. Having subsequently established its occurrence in brewer's yeast, ox blood and horse liver, i.e. "in nature wherever all other members of the vitamin B complex exist", they named it vitamin B₁₅. On the basis of experimental evidence assembled, the product "appeared to be an amino derivative of glycuronic acid". Chemically it was referred to as the "dimethyl-aminoacetate of gluconic acid".

A derivative, designated as vitamin B₁₅H₈, was thereafter claimed to have been synthesized from *D*-gluconolactone, dichloroacetic acid and di-*iso*-propylamine. It was assigned Structure II and later depicted as Structure III (Fig. 1). Different authors referred to it loosely as vitamin B₁₅, Pangametin, Sopangamine or a pangamic acid homologue. To add further to the confusion surrounding the chemistry and nomenclature of the compound, even the reaction product of two of the starting materials in the synthesis of vitamin B₁₅H₈—di-*isopropylammonium* dichloroacetate (Structure IV, Fig. 1)—came also to be called vitamin B₁₅.²⁻⁴

Casu *et al.*⁵ examined several commercial vitamin B₁₅ preparations and concluded that these products should be regarded as mixtures composed of sodium gluconate, glycine and di-*isopropylammonium* dichloroacetate in variable proportions. Concerned about recent reports of the parenteral administration

of vitamin B₁₅ formulations to horses at Canadian race tracks, and the availability of such preparations for allegedly investigational, therapeutic or clinical use, we made a study of the composition of commercial samples of the chemical and of five pharmaceutical formulations obtained by officers of the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

MATERIALS

A. Commercial Sample

Product I was a specimen obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A. Its label stated: Pangamic Acid (Vitamin B₁₅). For Chemical and Investigational Use only.

B. Pharmaceutical Formulations

Product II consisted of capsules labelled as follows: Vitamin B₁₅, Pangametin. Each capsule contains 50 mg. crystalline sodium salt of D-(N-di-*isopropylamino*). For experimental use by qualified investigators only.

Product III—an injection—was labelled as follows: Vitamin B₁₅, Pangametin. Each c.c. contains 100 mg. crystalline sodium salt of D-(N-di-*isopropylamino*). For experimental use by qualified investigators only.

Product IV was an injection for which the following label claims were made: Sopangamine, Vitamin B₁₅ Injection. Each 10 c.c. contains N/Di-*isopropylamino* Acetate 50 mg.; Benzyl Alcohol 1.5%. For therapeutic and investigational use.

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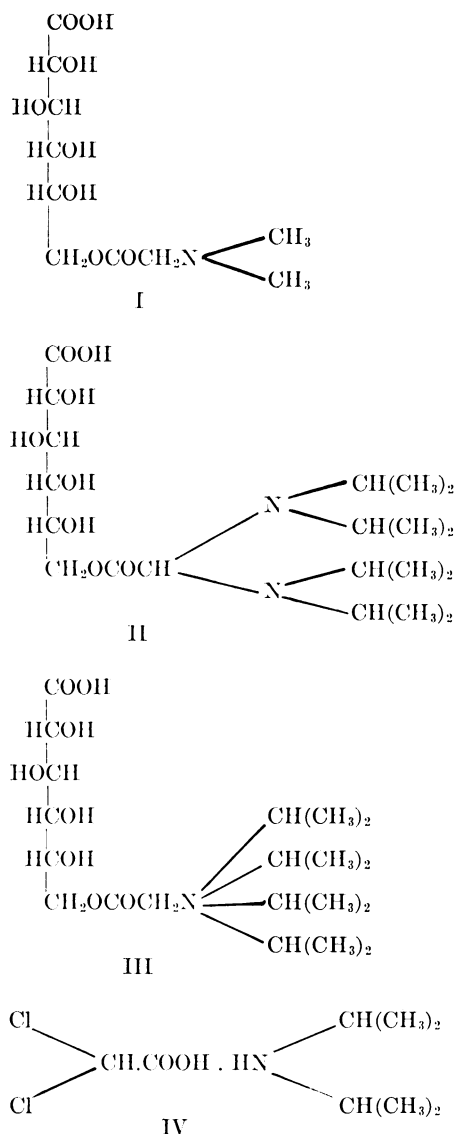


Fig. 1.—Formulas used to represent vitamin B₁₅.

Product V was an injection bearing the label: Sopangamine, Vitamin B₁₅ Injection. Each c.c. contains N-Diisopropylamino Acetate 10 mg.; Benzyl Alcohol 1.5%. For therapeutic and investigational use.

Product VI was an injection whose label read: Sopangamine, Vitamin B₁₅ Injection. Each c.c. contains N-Diisopropylamino Acetate 50 mg.; Benzyl Alcohol 1.5%. For therapeutic and investigational use.

ANALYTICAL METHODS FOR ESTABLISHING THE COMPOSITION OF EACH PRODUCT

Determination of Sodium Gluconate.—Each sample was extracted with ether, and the ether-insoluble residue—examined by infrared spectrophotometry—proved to be sodium gluconate. X-ray diffraction analyses confirmed this finding. Quantitative determinations were carried out by flame emission spectrophotometry. Liquid samples were

processed similarly following evaporation of 1 ml. aliquots to dryness.

Determination of Di-isopropylammonium Dichloroacetate.—The ether-soluble fraction of each sample was analyzed by infrared spectrophotometry and found to contain di-isopropylammonium dichloroacetate. X-ray diffraction measurements confirmed the spectral identification. Quantitative results were deduced from the amount of steam-volatile nitrogen generated on boiling with alkali.

Determination of Glycine.—The presence or absence of the amino acid in each sample was determined by thin-layer chromatography, using chromatoplates coated with silica gel-G. The solvent system used was butanol:acetone:water:acetic acid mixed in a ratio of 100:40:80:10 by volume. The spray reagent consisted of a mixture (50:3) of ninhydrin solution [0.1 g. of ninhydrin in ethyl alcohol:acetic acid:2,4,6-collidine (50:10:2)] and copper nitrate (1%) in ethyl alcohol, freshly prepared before use. Glycine, if present, appeared as a brown-orange colouration (R_f of 0.37) on a white background upon spraying with the ninhydrin reagent. Quantitative results were derived from total and steam-volatile nitrogen determinations and confirmed by non-aqueous titrimetry of the ether-soluble and ether-insoluble fractions.

Determination of Benzyl Alcohol.—The presence of benzyl alcohol, claimed to be contained in some of the products, was established by means of thin-layer chromatography, using chromatoplates coated with silica gel-G impregnated with paraffin oil. The solvent system used was a mixture of methanol and water in a ratio of 70:30 by volume. Phosphomolybdic acid (5%) in ethyl alcohol served as spray reagent. Benzyl alcohol appeared as a light-blue colouration (R_f of 0.63) on a dark-blue background following application of the spray reagent. Quantitative data were obtained by ultraviolet spectrophotometry: for benzyl alcohol, λ_{\max} . 256 $m\mu$ and $E_{1\%}^{1\text{cm}}$. 18.6.

RESULTS AND DISCUSSION

Experimental results obtained are shown in Table I. In accord with observations made by Casu *et al.*⁵ they illustrate that pangamic acid (vitamin B₁₅) is not a compound but a mixture of two or three compounds (Product I). Its melting point, suggested to be 165 (± 5) °C. (decomp.)⁶—the same as that reported for vitamin B₁₅H₈⁴—was found to range from 130 to 180 °C., indicating the presence of an impure compound or a mixture of compounds. The recognition of distinctly different crystal forms under the microscope supported the latter interpretation.

The composition of Product II differed markedly from that of the commercial specimen (Product I). While both preparations contained the same amount of glycine, their di-isopropylammonium dichloroacetate:sodium gluconate ratios were reversed. In product III this ratio was more like that

TABLE I.—EXPERIMENTAL RESULTS

Constituent	Product*						
	I	II		III	IV	V	VI
	% wt./wt.	mg./caps.	% wt./wt. as vitamin B ₁₅	mg./ml.	mg./ml.	mg./ml.	mg./ml.
Di-isopropylammonium dichloroacetate.....	57	9.4	40	66	4.9	4.9	5.0
Sodium gluconate.....	29	11.0	46	42	5.6	5.2	5.3
Glycine.....	14	3.3	14	0	0	0	0

*For label claims see Experimental Section.

of the commercial specimen, but this product contained no glycine. For three of the injectable solutions (products IV, V and VI) the di-isopropylammonium dichloroacetate: sodium gluconate ratio was similar to that of Product II but appreciably different from that of Product III and that of the commercial specimen. Microchemical tests for glycine were negative (see Addendum). Evidently, the products represent different compositions and, moreover, formulations which are at variance with label claims, owing to lack of proper pharmaceutical quality control.⁷

Numerous physiological and pharmacological properties have been attributed to pangamic acid, its homologues and analogues, on the basis of short-term animal experiments. These products have been reported to facilitate oxygen uptake, counteract cellular hypoxia, increase hemoglobin content and circulatory efficiency, normalize liver metabolism, exhibit neuromuscular blocking activity, induce hypotension, stimulate the hypophyseal-adrenal system, increase creatinine production in muscle tissues and display anti-rheumatic activity.⁸⁻²³ It appears that, in many of the experiments, preparations were used without prior physicochemical characterization and adequate knowledge of their composition. The interpretation of physiological and pharmacological effects thus obtained is highly suspect, and their extrapolation to clinical responses unjustified and unwarranted.²⁴

So far not a single critical study demonstrating any therapeutic usefulness of pangamic acid has been presented to the Food and Drug Directorate. Until experimental evidence is advanced which demonstrates that the product is a definite chemical entity, safe and likely to be effective for the purposes claimed, its distribution in Canada, even to clinical investigators, is prohibited. Its sale as a commercial medicinal preparation constitutes an even more serious offence.

ADDENDUM

After this manuscript was submitted for publication a product named CARDIOBEE 15 was also studied. The manufacturers claim that it contains di-isopropylammonium dichloroethanoate (100 mg. per 10 cc.) and benzyl alcohol (2%) in physiological saline solution. The product label stated "For Veterinary Use Only" and suggested that the product be administered in a dose of "5 cc. twice weekly for vitamin B-15 deficiency in horses". Qualitative analyses showed that, unlike any of the injectables examined previously (Table I), the preparation contained glycine in addition to di-isopropylammonium dichloroacetate and sodium gluconate.

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REFERENCES

1. KREBS, E. T., SR. *et al.*: *Int. Rec. Med.*, **164**: 18, 1951.
2. BEARD, H. H. AND WOFFORD, G.: *Exp. Med. Surg.*, **14**: 169, 1956.
3. KREBS, E. T. AND KREBS, E. T., JR.: N-substituted glycine esters of gluconic acid, U.S. Patent 2,710,876, June 14, 1955.
4. HARRIS, A. T.: The science of transmethylation and methylating agents, Ph.D. thesis; The University of Edinburgh, Edinburgh, Scotland, 1960.
5. CASU, B. *et al.*: *Boll. Chimicofarm.*, **97**: 3, 1958.
6. Nutritional Biochemicals Corporation: Personal communication.
7. LEVI, L., WALKER, G. C. AND PUGSLEY, L. I.: *Canad. Med. Ass. J.*, **91**: 781, 1964.
8. NAKANISHI, N. *et al.*: *J. Jap. Biochem. Soc.*, **32**: 235, 1960.
9. DOKUKIN, A. V. *et al.*: *Dokl. Akad. Nauk S.S.S.R.*, **144**: 675, 1962.
10. UDALOV, Y. F.: *Ibid.*, **143**: 734, 1962.
11. AIZAWA, T. AND HAMAYA, S.: *Jap. Heart J.*, **2**: 133, 1961.
12. IZQUIERDO, M., AGUIRRE, D. AND VALENZUELA, M. A.: *Arch. Inst. Farmacol. Exp. (Madrid)*, **11**: 102, 1959.
13. NAVARRO, M. D.: *S. Tomas J. Med.*, **9**: 376, 1954.
14. MARSHALL, F. N., ADAMSON, R. H. AND LONG, J. P.: *Proc. Soc. Exp. Biol. Med.*, **107**: 420, 1961.
15. SESE, B. *et al.*: *S. Tomas J. Med.*, **14**: 97, 1959.
16. SANTI, P.: *Med. Sper.*, **34**: 203, 1958.
17. BERTELLI, A. AND CASENTINI, S.: *Boll. Soc. Ital. Biol. Sper.*, **33**: 885, 907, 1957.
18. DETTEMANTE, A.: *Monit. Farm. (Madrid)*, **64**: 145, 1958.
19. CASENTINI, S. AND VILLANI, R.: *Atti Soc. Lombarda Sci. Med.-Biol.*, **12**: 435, 1957.
20. MASCITELLI-CORIANIOLI, E., BOLDRINI, R. AND CITTERIO, C.: *Naturwissenschaften*, **45**: 190, 1958.
21. BERTELLI, A., CASENTINI, S. AND TESSARI, L.: *Atti Soc. Lombarda Sci. Med.-Biol.*, **12**: 403, 1957.
22. BERTELLI, A., CASENTINI, S. AND LANZETTA, A.: *Minerva Med.*, **2**: 3425, 1957.
23. BERTELLI, A. AND CASENTINI, S.: *Boll. Soc. Ital. Biol. Sper.*, **34**: 1532, 1958.
24. LEVI, L. *et al.*: *Canad. Med. Ass. J.*, **92**: 1057, 1965.

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ONLY THREE HOSPITALS . . .

To obtain the greatest value from dietetics, it is important that exact measurements and methods should be employed, and these until recently have been wanting in our hospitals. A writer in "Modern Hospital" for 1914, stated as a fact that there were only three hospitals at that date in the United States in which an exacting physician could count on having his patient placed on an accurate and scientific-

cally arranged dietary for any considerable period of time, and in which a nurse could tell the physician exactly what his patients were getting in terms of the various elementary constituents of a dietary. The Massachusetts General Hospital was the pioneer in rendering a practical system of scientific feeding possible in general hospitals and in so doing has again placed the medical profession in its debt, —Editorial, *Canad. Med. Ass. J.*, **6**: 432, 1916,